

- (ii) a test compound; and
 - (b) detecting activation of the *hedgehog* pathway;
- wherein a statistically significant change in the activation of the *hedgehog* pathway in the presence of the test compound, relative to the activation in the absence of the test compound, indicates a *hedgehog*-mimicking activity for the test compound.

- 3. **(Reiterated)** The assay of claim 1, wherein the reaction mixture comprises a cell including a heterologous nucleic acid recombinantly expressing the *patched* receptor.
- 4. **(Reiterated)** The assay of claim 1, wherein detecting activation of the *hedgehog* pathway comprises observing a phenotype of a cell in the presence and absence of the test compound.
- 5. **(Reiterated)** The assay of claim 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of an intracellular second messenger responsive to signaling by the *patched* polypeptide.
- 6. **(Reiterated)** The assay of claim 3, wherein detecting activation of the *hedgehog* pathway comprises detecting a change in the level of expression of a gene controlled by a transcriptional regulatory sequence responsive to signaling by the *patched* polypeptide.
- 7. **(Reiterated)** The assay of claim 3, wherein the recombinant cell substantially lacks expression of an endogenous *patched* receptor.

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17. **(Amended)** An assay for screening test compounds to identify agents that mimic a bioactivity of a *hedgehog* protein, comprising:
- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
 - ii. contacting the cell with a test compound; and
 - iii. detecting activation of the *hedgehog* pathway,
- wherein a statistically significant change in the level of activation of the *hedgehog* pathway is indicative of an agent that mimics a bioactivity of a *hedgehog* protein.

18. (Amended) The assay of claim 17, wherein the activation of the *hedgehog* pathway is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

19. (Amended) The assay of claim 18, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

20. (Amended) The assay of claim 17, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by activation of the *hedgehog* pathway, and wherein expression of the reporter gene provides a signal for detecting activation of the *hedgehog* pathway.

21. (Reiterated) The assay of claims 20 or 33, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, and drug resistance.

22. (Reiterated) The assay of claim 21, wherein the reporter gene encodes a gene product selected from chloramphenicol acetyl transferase, luciferase, betagalactosidase, and alkaline phosphatase.

23. (Reiterated) The assay of claim 20, wherein the reporter gene includes a transcriptional regulatory sequence of a gene selected from a *GLI* gene and *patched* gene.

24. (Amended) The assay of claim 17, wherein activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to activation of the *hedgehog* pathway.

25. (Amended) The assay of claim 24, wherein the activation of the *hedgehog* pathway is detected by changes in intracellular protein phosphorylation.

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26. (Amended) The assay of claim 17, wherein the receptor is a *patched* protein.

27. (Reiterated) The assay of claim 17 or 26, wherein the cell further comprises a heterologous gene construct encoding the receptor.

29. (Reiterated) The assay of claim 17, wherein the cell further comprises one or more heterologous gene constructs encoding *costal-2*, *fused* and/or *smoothened* genes, or homologs thereof.

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30. (Amended) An assay for screening test compounds to identify agents that activate hedgehog signal transduction, comprising:

- i. providing a cell having a recombinant expression vector encoding a naturally occurring mammalian *patched* protein;
- ii. contacting the cell with a test compound under conditions wherein the *patched* protein is expressed; and
- iii. detecting a change in hedgehog signal transduction,

wherein a statistically significant change in hedgehog signal transduction in the presence of the test compound, relative to in the absence of the test compound, is indicative of an agent that activates hedgehog signal transduction.

31. (Amended) The assay of claim 30, wherein the signal transduction is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

32. (Reiterated) The assay of claim 30, wherein the cell is a human cell.

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33. (Amended) The assay of claim 30, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by hedgehog signal transduction, and wherein expression of the reporter gene provides a detectable signal for detecting hedgehog signal transduction.

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34. (Amended) The assay of claim 1, 15, 26 or 30, wherein the *patched* protein is of vertebrate origin.

35. (Amended) The assay of claim 34, wherein the *patched* protein is a mammalian *patched* protein.

36. (Amended) The assay of claim 35, wherein the *patched* protein is a human *patched* protein.

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42. (Amended) The assay of any of claims 3, 17, 30, or 78, wherein the cell is a metazoan cell.

43. (Reiterated) The assay of claim 42, wherein the cell is a mammalian cell.

44. (Reiterated) The assay of claim 42, wherein the cell is an insect cell.

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45. (Amended) The assay of any of claims 3, 17, 30, or 78, wherein the cell is a oocyte.

46. (Amended) The assay of any of claims 3, 17, 30, or 78, wherein the cell is a yeast cell.

47. (Amended) The assay of claim 1, 8, 17, 30, or 78, wherein the steps of the assay are repeated for a variegated library of at least 100 different test compounds.

48. (Amended) The assay of claims 1, 8, 17, 30, or 78, wherein the test compound is selected from small organic molecules and natural product extracts.

49. (Amended) The assay of claim 1, 8, 17, 30, or 78, further comprising preparing a pharmaceutical preparation of one or more compounds identified.

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63. (Amended) A method for identifying *hedgehog* agonists, comprising:

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contacting a test agent with cells expressing a *patched* protein, wherein said cells undergo a detectable response when contacted with a naturally occurring *hedgehog* protein; and comparing the response of said cells to the test agent with the response of similar cells to a naturally occurring *hedgehog* protein.

64. (Reiterated) A method of claim 63, wherein said detectable response comprises expression of a gene controlled by a transcriptional regulatory sequence responsive to *patched*-mediated *hedgehog* signaling.

65. (Reiterated) A method of claim 64, wherein said detectable response comprises expression of secondary signaling molecules selected from Bmp-2, Bmp-4, and Fgf-4.

CG 66. (Amended) A method of claim 63, wherein said cells are transfected to express a recombinant form of the *patched* protein.

67. (Reiterated) A method of claim 63 or 66, wherein said cells are eukaryotic.

68. (Reiterated) A method of claim 63, 66, or 67, wherein said cells are vertebrate cells.

69. (Reiterated) A method of claim 63, 66, or 67, wherein said cells are mammalian cells.

70. (Reiterated) A method of claim 63, wherein said cells further comprise a reporter gene construct operably linked to a transcriptional regulatory element responsive to *hedgehog* signaling, and said detectable response comprises detecting the level of expression of said reporter gene, and comparing the response of said cells to a test agent.

71. (Reiterated) A method of claim 70, wherein expression of the reporter gene is detected by determining the protein product encoded by the reporter gene.

72. (Reiterated) A method of claim 71, wherein the reporter gene product is detected by an intrinsic activity associated with that product.

73. (Reiterated) A method of claim 71, wherein the reporter gene product is detected by an enzymatic activity associated with that product.

09 74. (Amended) A method of claim 63 or 70, wherein the transcriptional regulatory element is derived from target genes selected from the group consisting of *GLI*, *patched*, *cubitus interruptus*, and *fused*.

75. (Reiterated) A method of claim 63 or 70, wherein the detectable response comprises expression of homeobox genes.

76. (Reiterated) A method of claim 75, wherein the homeobox gene is *Hoxd*.

◇◇ Please add the following new claims:

710 78. (NEW) An assay for screening test compounds to identify agents that antagonize a bioactivity of a *hedgehog* protein, comprising:

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. detecting activation of the *hedgehog* pathway in said cell;
- iii. contacting the cell with a test compound;
- iv. detecting activation of the *hedgehog* pathway in said cell in the presence of said test compound,

wherein a statistically significant decrease in the level of activation of the *hedgehog* pathway in the presence of said test compound in comparison to the absence of said test compound is indicative of an agent that antagonizes a bioactivity of a *hedgehog* protein.

79. (NEW) The assay of claim 78, wherein the activation of the *hedgehog* pathway is detected by detecting a change in phenotype of the cell in the presence of the test compound.

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80. (NEW) The assay of claim 79, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

81. (NEW) The assay of claim 78, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by activation of the *hedgehog* pathway, and wherein expression of the reporter gene provides a signal for detecting activation of the *hedgehog* pathway.

82. (NEW) The assay of claim 81, wherein the reporter gene encodes a gene product that gives rise to a detectable signal selected from color, fluorescence, luminescence, cell viability, relief of a cell nutritional requirement, cell growth, and drug resistance.

83. (NEW) The assay of claim 82, wherein the reporter gene encodes a gene product selected from chloramphenicol acetyl transferase, luciferase, betagalactosidase, and alkaline phosphatase.

84. (NEW) The assay of claim 81, wherein the reporter gene includes a transcriptional regulatory sequence of a gene selected from a *GLI* gene and *patched* gene.

85. (NEW) The assay of claim 78, wherein activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to activation of the *hedgehog* pathway.

86. (NEW) The assay of claim 85, wherein the activation of the *hedgehog* pathway is detected by changes in intracellular protein phosphorylation.

87. (NEW) A method of manufacturing an agent that activates *hedgehog* signal transduction, comprising

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. contacting the cell with a test compound;

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- iii. detecting activation of *hedgehog* signal transduction, wherein a statistically significant change in the level of activation of *hedgehog* signal transduction is indicative of an agent that mimics a bioactivity of a *hedgehog* protein; and
- iv. synthesizing said compound so identified as an agent that mimics a bioactivity of a *hedgehog* protein.

88. (NEW) A method of manufacturing an agent that antagonizes *hedgehog* signal transduction, comprising

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. detecting activation of *hedgehog* signal transduction in said cell;
- iii. contacting the cell with a test compound;
- iv. detecting activation of *hedgehog* signal transduction in said cell in the presence of said test compound, wherein a statistically significant decrease in the level of activation of *hedgehog* signal transduction in the presence of said test compound in comparison to the absence of said test compound is indicative of an agent that antagonizes *hedgehog* signal transduction; and
- iv. synthesizing said compound so identified as an agent that antagonizes *hedgehog* signal transduction.

89. (NEW) The method of claim 87 or 88, further comprising formulating said agent in a pharmaceutically acceptable carrier.

90. (NEW) An agent that antagonizes *hedgehog* signal transduction identified by the assay of claim 78.

91. (NEW) An agent that activates *hedgehog* signal transduction identified by the assay of any of claims 17, 30 or 63.

The amended claims are re-stated below to reflect changes with respect to the last filing.

17. (Amended) An assay for screening test compounds to identify agents that mimic a bioactivity of a *hedgehog* protein, comprising:

- i. providing a cell expressing a *hedgehog* receptor, wherein said *hedgehog* receptor binds a naturally occurring *hedgehog* polypeptide;
- ii. contacting the cell with a test compound; and
- iii. detecting activation of the *hedgehog pathway receptor*,

wherein a statistically significant change in the level of activation of the *hedgehog pathway receptor* is indicative of an agent that mimics a bioactivity of a *hedgehog* protein.

18. (Amended) The assay of claim 17, wherein the activation of the *hedgehog pathway receptor* is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

19. (Amended) The assay of claim ~~18~~17, wherein the change in phenotype is detected by detecting gain or loss of expression of a cell-type specific marker.

20. (Amended) The assay of claim 17, wherein the ~~receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and~~ the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by activation of the *hedgehog pathway receptor*, and wherein expression of the reporter gene ~~providing~~ provides a detectable signal for detecting activation of the *hedgehog pathway receptor*.

24. (Amended) The assay of claim 17, wherein ~~the receptor transduces a signal in the cell which is sensitive to *hedgehog* binding, and~~ activation of the *hedgehog* receptor is detected by detecting change in a level of an intracellular second messenger responsive to activation of the *hedgehog pathway* signaling by the receptor.

25. (Amended) The assay of claim 24, wherein the activation of the hedgehog pathway receptor is detected by changes in intracellular protein phosphorylation.

26. (Amended) The assay of claim 17, wherein the receptor is a patched protein receptor.

30. (Amended) An assay for screening test compounds to identify agents that activate hedgehog signal transduction ~~a naturally occurring mammalian patched receptor~~, comprising:

- i. providing a cell having a recombinant expression vector encoding a naturally occurring mammalian patched protein receptor;
- ii. contacting the cell with a test compound under conditions ~~whereunder~~ wherein the patched protein is expressed; and
- iii. detecting ~~an effect, if any, of the test compound on~~ a change in hedgehog signal transduction by the patched protein,

wherein a statistically significant change in the hedgehog signal transduction of the patched receptor in the presence of the test compound, relative to in the absence of the test compound ~~or the patched receptor~~, is indicative of an agent that activates hedgehog signal transduction ~~modulates the activity of patched receptor~~.

31. (Amended) The assay of claim 30, wherein the signal transduction ~~by the patched receptor~~ is detected by detecting a change in phenotype of the cell relative to in the absence of the test compound.

33. (Amended) The assay of claim 30, wherein the cell further comprises a reporter gene construct comprising a reporter gene in operable linkage with a transcriptional regulatory sequence sensitive to intracellular signals transduced by hedgehog signal transduction, and wherein ~~interaction of a hedgehog polypeptide with the patched receptor~~, expression of the reporter gene ~~providing~~ provides a detectable signal for detecting hedgehog signal transduction ~~by the patched receptor~~.

34. (Amended) The assay of claim 1, 15, 26 or 30, wherein the patched protein receptor is of vertebrate origin.

35. (Amended) The assay of claim 34, wherein the *patched* protein receptor is a mammalian *patched* protein receptor.

36. (Amended) The assay of claim 35, wherein the *patched* protein receptor is a human *patched* protein receptor.

42. (Amended) The assay of any of claims 3, 17, ~~or 30~~, or 78, wherein the cell is a metazoan cell.

45. (Amended) The assay of any of claims 3, 17, ~~or 30~~, or 78, wherein the cell is a oocyte.

46. (Amended) The assay of any of claims 3, 17, ~~or 30~~, or 78, wherein the cell is a yeast cell.

47. (Amended) The assay of claim 1, 8, 17, ~~or 30~~, or 78, wherein the steps of the assay are repeated for a variegated library of at least 100 different test compounds.

48. (Amended) The assay of claims 1, 8, 17, ~~or 30~~, or 78, wherein the test compound is selected from small organic molecules and natural product extracts.

49. (Amended) The assay of claim 1, 8, 17, ~~or 30~~, or 78, further comprising preparing a pharmaceutical preparation of one or more compounds identified.

63. (Amended) A method for identifying *hedgehog* agonists, comprising:
contacting a test agent with cells expressing a *patched* protein receptor, wherein said cells undergo a detectable response when contacted with a naturally occurring *hedgehog* protein, ~~which response is dependent on expression of the *patched* receptor~~; and
comparing the response of said cells to the test agent with the response of similar cells to a naturally occurring *hedgehog* protein.